CLAIMS

We Claim:

1. A method for detecting RNA-dependent polymerase activity comprising:

(a) providing a primer oligonucleotide having a 3' OH;

(b) contacting said primer oligonucleotide with a template polynucleotide and allowing hybridization to occur to form a hybridized polynucleotide;

(c) adding an RNA-dependent polymerase to said hybridized polynucleotide to produce a mixture;

(d) adding a PP_i detection mixture to said mixture;

(e) adding a substrate mixture comprising a nucleotide triphosphate or an analog thereof to said mixture; and

(f) measuring a product of the PP_i detection mixture;

wherein

apyrase is not part of the mixture and

steps (c), (d) and (e) may be performed simultaneously or separately in any order.

2. The method of claim 1, wherein said RNA-dependent polymerase is a viral RNA-dependent RNA polymerase (RdRp) selected from the group consisting of Hepatitis C virus, poliovirus, West Nile virus, Dengue virus, Human T Cell Leukemia virus, St. Louis Encephalitis virus, Yellow Fever virus and Measles virus.

3. The method of claim 2, wherein said RdRp is from Hepatitis C virus.

4. The method of claim 1, wherein said RNA-dependent polymerase is an RNA-dependent DNA polymerase (RdDp).

5. The method of claim 4, wherein said RdDp is reverse transcriptase from Human Immunodeficiency virus.

6. The method of claim 1, wherein said hybridized polynucleotide comprises synthetic poly(A) and poly(U).

7. The method of claim 1, wherein said hybridized polynucleotide comprises synthetic poly(G) and poly(C).

8. The method claim 1, wherein said primer oligonucleotide and said template polynucleotide are on the same RNA molecule.

9. The method of claim 1, wherein said PP_i detection mixture comprises luciferase, luciferin, ATP sulphurylase and adenosine 5'-phosphosulfate (APS) and said product is emitted light.

10. The method of claim 9, wherein the emitted light is measured with a luminometer.

11. The method of claim 9, wherein said luciferase is a thermostable luciferase.

12. A method for evaluating an inhibitor of an RNA-dependent polymerase comprising:

(a) providing a primer oligonucleotide having a 3' OH;

(b) contacting said primer oligonucleotide with a template polynucleotide and allowing hybridization to occur to form a hybridized polynucleotide;

(c) adding an RNA-dependent polymerase to said hybridized polynucleotide to produce a mixture;

(d) adding a PP_i detection mixture to said mixture;

(e) adding a substrate mixture comprising a nucleotide triphosphate or an analog thereof to said mixture;

(f) adding a compound that is or is suspected of being an inhibitor of said RNA-dependent polymerase; and

(g) measuring a product of the PP_i detection mixture;

wherein

apyrase is not part of the mixture, and

steps (c), (d), (e) and (f) may be performed simultaneously or separately in any order.

13. The method of claim 12, wherein said RNA-dependent polymerase is a viral RNA-dependent RNA polymerase (RdRp) selected from the group consisting of Hepatitis C virus, poliovirus, West Nile virus, Dengue virus, Human T Cell Leukemia virus, St. Louis Encephalitis virus, Yellow Fever virus and Measles virus.

14. The method of claim 13, wherein said RdRp is a recombinantly produced Hepatitis C virus NS5B.

15. The method of claim 12, wherein said RNA-dependent polymerase is an RNA-dependent DNA polymerase (RdDp).

16. The method of claim 15, wherein said RdDp is reverse transcriptase from Human Immunodeficiency virus.

17. The method of claim 12, wherein said hybridized polynucleotide comprises synthetic poly(A) and poly(U).

18. The method of claim 12, wherein said hybridized polynucleotide comprises synthetic poly(G) and poly(C).

19. The method claim 12, wherein said primer oligonucleotide and said template polynucleotide are on the same RNA molecule.

20. The method of claim 12, wherein said PP_i detection mixture comprises luciferase, luciferin, ATP sulphurylase and adenosine 5'-phosphosulfate (APS) and said product is emitted light.

21. The method of claim 20, wherein the emitted light is measured with a luminometer.

22. The method of claim 21, wherein said luciferase is a thermostable luciferase.